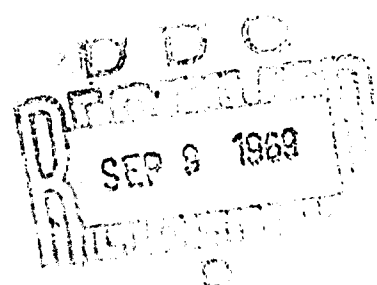
TRANSLATION NO. 2478

DATE: 14 Aug 1969



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PATHOGENESIS AND MORPHOLOGICAL CHANGES IN THE LUNGS OF MICE DURING EXPERIMENTAL INFECTION WITH THE PARAINFLUENZA SENDAI VIRUS

[Following is the translation of an article by N. A. Maksimovich and Hou Yun'-de, Institute of Infectious Diseases, AMN USSR, Kiev, and the Institute of Virology imeni D. I. Ivanovskiy, AMN USSR, Moscow, published in the Russian-language periodical Voprosy Virusologii (Problems of Virology) No 7, 1962, pages 168--174. It was submitted on 27 Feb 1961.]

Recently, in agreement with the nomenclature of Andrews and associates [10], the type D influenza virus has been related to the parainfluenza viruses type I, subtype Sendai.

Up until the present the morphological changes which are caused by this virus during experimental infection have still been studied little. Also still unclear are the differences in changes in the upper respiratory passages and lungs of mice which are caused by the influenza virus and the Sendai virus. Considered as characteristic for the latter are focal necroses of the epithelium of the trachea and bronchi which are accompanied by the development of pneumonia [21]. Noda [21] was not able to detect either viral inclusions or elementary bodies in cells of bronchial epithelium of mice which were infected with the Sendai virus.

Tanaka and associates [26] observed an expressed proliferative reaction of the chorioallantoic membrane of chick embryos after infection with the Sendai virus. Yukino [29] and V. N. Tarasov [8] obtained similar data while studying changes in the lungs of mice, and they, as well as V. Ye. Pigarevskiy [6], observed oxyphilic bodies and basophilic inclusions, which are characteristic for influenza, in the epithelium of the trachea and bronchi already in 48 hours after the infection of mice and up to 5 days, when the majority of mice perished. By this time there were noted in the lungs the symptoms of hemorrhagic edema with infiltration of the interalveolar septa by mononuclear cells and a large number of leukocytes in the lumina of the bronchi.

As regards the accumulation of hemagglutinins in the lungs of mice which were infected with the Sendai virus, then some investigators [1, 29] noted a positive reaction of hemagglutination with a suspension of pulmonary tissue of infected mice, and others [5, 8, 17] did not detect hemagglutinating properties in suspensions from the lungs of infected mice.

Though all the cited works were carried out on the Japanese strain of the Sendai virus, the authors, as it follows from the

findings presented, arrived at quite contradictory results.

The present investigation was carried out on 2 strains of the Sendai virus (Japanese and Vladivostok variants). In particular our mission included a study of the distribution of Sendai virus in the internal organs of mice following intranasal infection.

Materials and Methods

The tests were carried out on 200 white mice weighing 9-11 g. The mice were infected in the nose under a light ether narcosis with fresh allantoic fluid containing the virus; the dose was 0.03 ml for each mouse. We used freshly isolated strains of the virus after 3-6 passages in a chick embryo: LM-1 (Vladivostok variant), isolated from mice in Moscow in 1959, and Ufa (Japanese variant), isolated from man in Ufa in 1959 [9]. Allantoic cultures of virus were obtained by means of infection of 11-day old chick embryos in the allantoic cavity. The eggs were opened after 48 hours of cultivation at 37°. Titers of virus on chick embryos were 10^{-7} and 10^{-8} (ID_{50}), and on mice for the LM-1 strain 10^{5}, $10^{-6.5}$, and for the Ufa strain 10^{-3}, 10^{-5} (LD_{50}).

Four mice each were sacrificed in periods of 6, 24, 48, 72, 96, and 120 hours after infection. At the same time noninfected mice were investigated for a control. For autopsy blood was taken from the jugular vein and then a 10% suspension was prepared from the brain, lungs, trachea, liver, spleen, pancreas, kidneys, and muscles of the haunch on physiological solution for titration of the virus on chick embryos and the hemagglutination reaction (HR). Part of the organs were sealed in 10% formalin solution for histological investigation.

The degree of damage to the lungs of mice was expressed in percentages by the method of Ginsberg and Horsfall [14]. The entire process of infection and autopsy of mice was carried out in a table isolation box.

Infectious titer of ID_{50} was determined by means of infection of 11-day old chick embryos in the allantoic cavity with 10-fold dilutions of materials. Results were considered by the method of Reed and Muench [23].

The HR was set up on plates made of Plexiglas with a 1% suspension of chicken erythrocytes at room temperature. The results of the reaction were considered in 45 minutes.

Histological preparations were prepared by the generally accepted methods: they were sealed in celloidin-paraffin, stained

with hematoxylin-eosin and by Van Gieson's method; after desiccation and clearing the stained sections were enclosed in Canadian balsam.

Results of Investigations

Accumulation of virus in the organs of mice which were infected intranasally with the Sendai parainfluenza virus.

Mice were infected with the LM-1 strain in doses of 1000 LD_{50}. The titer of the virus was determined in various organs by means of infection of chick embryos. The results are presented in Table 1, from which it can be seen that after 24 hours from the time of infection the virus was already multiplying considerably in the lungs of the mice, and after 72 hours the titer of virus was established at a maximum level ($10^{-7.8}$); on the 4th and 5th days after infection the titer of virus was somewhat reduced (to $10^{-6.3}$--$10^{-6.0}$).

Table 1

Distribution of Sendai parainfluenza virus (strain LM-1) in the organs of mice after intranasal infection with doses of 1000 LD_{50}

Орган (a)	Время после заражения (часы) (b)						
	0	6	24	48	72	96	120
c Кровь[1]	0	0	0—2,7	0	0	0—1	0
d Мозг[1]	0	0	0	0	0—2,3	0	0—1,5
e Сердце[1]	0	0	0—1	1—1,5	0—1,5	1,5—2,5	1,5—2,5
f Легкие	0	1,5	≥1,3	5,5	7,3	6,3	6
g Трахея	0	0	0	0	0	0	0
h Печень[1]	0	0	0	0	0—1,5	1	0
i Селезенка	0	0	0	0	0	0	0
j Поджелудочная железа	0	0	0	0	0	0	0
k Почка	0	0	0	0	0	0	0
l Мышца	0	0	0	—	0	—	0

1 - Test repeated 3 times.

Legend: Figures denote the logarithm of infectious titer on embryos (ID_{50}); 0-infectious titer less than 1:10, for the trachea less than 1:20.

Key: (a) Organ; (b) Time after infection (hours); (c) Blood; (d) Brain; (e) Heart; (f) Lungs; (g) Trachea; (h) Liver; (i) Spleen; (j) Pancreas; (k) Kidneys; (l) Muscle.

On the 3rd day after infection the symptoms of illness appeared in the mice: cough, lessening of activity, fraying of fur; on the 4th day panting began, and on the 5th day all the mice died.

In addition to the lungs the virus was detected irregularly in an insignificant titer in the brain, heart, and liver. In 24 and 96 hours after infection viremia was noted.

The virus was not detected in the trachea, spleen, pancreas, kidneys, and muscles of the haunch.

Table 2 presents the results of a comparison of the infectious titer ID_{50} and the titer of hemagglutinins (HA) with damage to the lungs. As can be seen from the table, hemagglutinins appear in later stages of infection - on the 3rd day after infection, in spite of the fact that on the 1st and 2nd day after infection the infectious titer already reaches a high level ($10^{-7.5}$); damage to the lungs is revealed almost simultaneously with the appearance of hemagglutinins.

Table 2

Dynamics of multiplication of the parainfluenza Sendai virus (Japanese and Vladivostok variants) in the lungs of mice (dose of infection 1000 LD_{50})

Штамм (a)	Показатель (b)	Время после заражения (часы) (c)						
		0	0	24	48	72	96	120
Владивостокский вариант (d)								
ЛМ-1 (f)	lg ID_{50}	0	1,5	4,3	5,5	7,33	6,3	6,0
	Титр НА (h)	0	<1:4	<1:4	<1:4	1:16	1:8	1:1 024
	Поражение легких (в %) (i)	0	0	0	0	2	60	100
Уфа (g)	lg ID_{50}	0	3,5	7,0	7,5	—	8,6	—
	Титр НА (h)	0	<1:4	<1:4	<1:4	—	1:64	—
	Поражение легких (в %) (i)	0	0	0	25	—	64	—
Японский вариант (e)								
ЛМ-1 (f)	lg ID_{50}	0	3,3	<4,5	6,3	5,3	5,3	7,3
	Титр НА (h)	0	<1:4	<1:4	<1:4	1:8	1:128	1:64
	Поражение легких (в %) (i)	0	0	0	0	0	45	60

Legend: Same as for Table 1.
Key: (a) Strain; (b) Index; (c) Time after infection (hours); (d) Vladivostok variant; (e) Japanese variant; (f) LM-1; (g) Ufa; (h) HA titer; (i) Damage to lungs (in %).

It is necessary to note that the virus detected in the heart differs from the virus detected in the lungs. As can be seen from Table 3, the coefficient of the ID_{50}/HA in the heart in the first days after infection is very low and then increases gradually, and

the coefficient of the ID_{50}/HA in the lungs, conversely, is very high at first and then is gradually lowered. These data indicate that after intranasal infection a virus is formed in the heart of mice which is characterized by a low infectious titer and a comparatively high titer of hemagglutinins, which is a sign of the formation of an incomplete virus.

Table 3

Dynamics of multiplication of parainfluenza virus (Sendai, strain LM-1) in the lungs and heart of mice (dose of infection 1000 LD_{50})

(a) Орган	(b) Показатель	(c) Время после заражения (в сутках)					
		0	1	2	3	4	5
(d) Сердце	[illegible]	0	1,0	1,5	0	2,5	2,5
	[illegible]	0	1,3	0,9	0,3	0,9	0,6
	[illegible]	0	—0,3	0,6	—	1,6	1,9
(e) Легкие	[illegible]	0	>6,5	7,75	7,0	6,0	6,0
	[illegible]	0	<0,3	0,3	0,6	1,8	2,1
	[illegible]	0	>6,2	7,15	6,4	4,2	3,9

Legend: Same as for table 1.
Key: (a) Organ; (b) Index; (c) Time after infection; (d) Heart; (e) Lungs.

Histopathological changes in the lungs of mice, infected intranasally with the LM-1 strain of the parainfluenza Sendai virus in doses of 1000 LD_{50}

In mice which were sacrificed 6 hours after infection a diffuse thickening is revealed in the interalveolar septa (Fig. 1) and the pleurae themselves due to expansion of the capillaries and infiltration, primarily by leukocytes and a small amount of lymphocytes. In places focal accumulations of leukocytes are observed. Around the small vessels and bronchi there are infiltrates of leukocytes and lymphocytes and the tissue surrounding them is edematous. Proliferation of the epithelim of the bronchi is observed and it is disposed in 2--3 layers, sometimes forming papillae. Basophilic inclusions and fuchsinophilic bodies are not revealed.

In mice which were sacrificed after 1-2 days plethora of the capillaries and minute vessels is expressed somewhat less, and in some places there are hemorrhages in the lumen of the alveoli. In certain bronchi a more expressed proliferation of the epithelium of the bronchi is observed, sometimes the latter almost completely

fill the lumen of the small bronchi (Fig. 2). Rose-colored homogeneous masses are disposed parietally in some alveoli. Minute cytoplasmatic inclusions are revealed in the epithelium of the bronchi. Along with inclusions the cells of alveoli and partially the epithelium of the bronchi contain clumps of hemosiderin.

Figure 1. Diffuse thickening of the interalveolar septa, pleurae, and subpleural sectors in the lungs of mice which were sacrificed 6 hours after infection. Staining with hematoxylin-eosin. Magnification 100X.

Figure 2. Papillary proliferation of the large bronchus in the lungs of a mouse which was sacrificed 2 days after infection; in the lumen of the bronchus cast-off cells of epithelium are observed. Staining with hematoxylin-eosin. Magnification 100X.

In mice which were sacrificed after 3 days, in addition to the above-described changes in the lungs minute foci of pneumonia are revealed and in them the lumina of the alveoli are filled with leukocytes, erythrocytes, and cast-off alveolar cells. In the pneumonia sectors the bronchi and vessels are surrounded by leukocytic infiltrates and in places minute hemorrhages are observed (Figure 3).

Figure 3. Small hemorrhage in the lungs of a mouse which was sacrificed after 3 days. Staining with hematoxylin-eosin. Magnification 100X.

In mice which were sacrificed after 4 days sectors of atelectasis are observed along with sectors of emphysema. Over a large stretch there is well expressed interstitial leukocytic infiltration, and also a small edema. The number of foci of pneumonia was somewhat increased. In the epithelium of the bronchi a small number of well-formed eosinophilic bodies is observed. They are of a pale-rose color (Fig. 4) and one or several dark punctate basophilic particles are visible in them. Large inclusions often force the nucleus of the cell to the periphery.

Figure 4. Lungs of a mouse which was sacrificed after 4 days. In the cell of bronchial epithelium there is a large inclusion which is a pale color and in which dark punctate particles are visible. The inclusion forces the cell nucleus to the periphery. Staining with hematoxylin-eosin. Magnification 400X.

In mice which were sacrificed after 5 days foci of pneumonia are revealed and in some they are widespread in nature. In the foci of pneumonia the lumina of the alveoli are filled with a large number of leukocytes, partially disintegrating, and also cast-off alveolar cells; the lumina of the bronchi, primarily the small ones, are filled with a profuse purulent exudate (Fig. 5). A share of the epithelial cells are found in a state of necrosis and casting off. The bronchi and vessels are surrounded by lympho-leukocytic infiltrates. Outside the sectors of pneumonia there are foci of atelectasis and emphysema, and well expressed leukocytic infiltration of the interalveolar septa in sectors of the lungs located under the pleurae. There are sectors of hemorrhages in the lumen of the alveoli. The alveolar cells contain much hemosiderin. In the epithelial cells of the bronchi and trachea large eosinophilic cytoplasmic inclusions are detected. Microbes are absent in all the preparations.

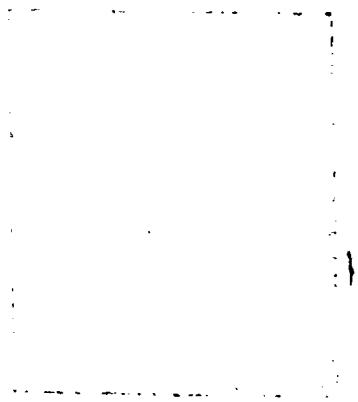

Figure 5. Focus of pneumonia in the lungs of a mouse which was sacrificed after 5 days. Staining with hematoxylin-eosin. Magnification 200X.

Consequently, along with the multiplication of the Sendai virus in the lungs of mice an inflammatory process develops which is characterized by interstitial, primarily leukocytic infiltration, foci of pneumonia, expressed proliferation of the bronchial epithelium, and vascular disorders. The latter are especially well expressed immediately after infection of the mice and on the 4th-5th day, when pneumonia is developed. In the lungs of infected mice there is a weakly expressed necrotic destructive bronchitis in comparison with infection with the influenza virus A-PR8 which is pathogenic for mice.

Discussion

After the intranasal infection of mice with the parainfluenza Sendai virus in doses of 1000 LD_{50} the virus not only multiplied easily in the lungs, but was also detected in the blood, heart, liver, and brain. Those data are similar with the data of other authors relative to the virus of influenza type A [7, 19, 27]. After intranasal infection a virus is formed in the heart which possesses a very low ID_{50}/HA coefficient, which indicates the formation of an incomplete virus in low-sensitivity tissue. The formation of an incomplete virus in internal organs (liver, kidneys) was described by Mizuno [19] following the infection of mice with type A influenza virus in the brain and abdominal cavity. On the 1st-3rd day after infection a large amount of infectious Sendai virus is formed in the lungs without the accumulation of hemagglutinins, in contrast to the influenza virus which is adapted to mice [3, 25].

A study of histopathological changes in the lungs after infection with a large dose of virus shows that by the moment of death of the mice (5 days after infection) there is a comparatively weakly expressed necrotic destructive bronchitis, which is characteristic for infection with the mouse-adapted viruses of influenza A, A_1, B, and swine [2-4, 11-13, 18, 20, 22, 24, 25].

In our experiments for reproducing acute infection in mice we used the LM-1 strain with a titer of lethality for mice of $10^{-6.5}$, which is the most pathogenic for these animals, and also the Japanese strain, with which other authors worked. The titer of lethality for the latter comprised 10^{-3}--10^{-4}. Pathomorphological changes, detected by us and other authors [8, 26, 29] when using both strains, turned out to be very similar, in spite of differences in their pathogenic activity.

We consider that the dominance of processes of proliferation and infiltration over processes of necrosis is a distinctive feature of the parainfluenza Sendai virus in a comparison of it with the influenza virus.

It is necessary to note that in pulmonary infiltrates of mice infected with the Sendai virus the amount of leukocytes is much greater than in pulmonary infiltrates of mice which were infected with the virus of influenza type A. Similar results were obtained by V. N. Tarasov on rats which were infected with the parainfluenza Sendai virus [8].

The inclusions which we observed in the epithelial cells are similar with the inclusions described by V. Ye. Pigarevskiy [5] and Harford and associates [15, 16] in the lungs of mice which were infected with the virus of influenza type A. According to our data,

based on type and process of formation of inclusions parainfluenza infection does not differ from influenza. At the same time we detected fuchsinophilic inclusions, described by V. Ye. Pigarevskiy [6] and V. N. Tarasov [8] in mice which were infected with the Sendai virus, only in the late stages of development of infection.

Conclusions

1. After the intranasal infection of mice with the parainfluenza virus (Sendai) in doses of 1000 LD_{50} the virus not only multiplied easily in the lungs, but was also detected in the blood, heart, liver, and brain; in the heart of mice an incomplete virus is formed; hemagglutinins are revealed in the lungs only in the later periods of development of infection.

2. Histopathological changes in the lungs of mice which were infected with the parainfluenza Sendai virus are characterized by comparatively weakly expressed necrotic destructive processes in the epithelium of the bronchi in comparison with infection by the adapted virus of influenza. At first there sets in a leukocytic infiltration of the wall of the bronchus and proliferation of the bronchi which acquires a polynuclear disposition. In the last days of life of the mice pneumonia with a leukocytic exudate in the alveoli develops.

3. Inclusion bodies, detected during parainfluenza infection with the Sendai virus in the cells of epithelium, trachea, bronchi, and alveoli, do not differ from the cytoplasmic inclusions which are described by a number of authors for influenza.

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